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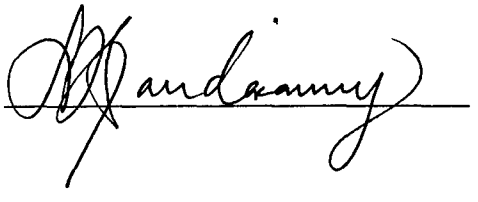

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13. ABSTRACT *(Maximum 200 Words)*

The proposed study seeks to address the interaction of environmental and genetic factors in the etiology of breast cancer. Cytochrome P450 isozyme (CYP1B1) metabolizes environmental and endogenously formed carcinogens in the breast. We are testing the hypothesis that individuals with higher levels of CYP1B1 are at a higher risk for breast cancer because they produce higher amounts of ultimate carcinogen. The expression level of *CYP1B1* is being determined in a collection of normal breast tissue samples from reduction mammoplasties and from mastectomy patients and *CYP1B1* expression is compared in specimen from cancer patients and healthy controls to establish if breast cancer patients have an increased level of the enzyme. During the last year a previously developed assay that uses reverse transcription (RT)-PCR to quantitate expression relative to the β-actin gene, was optimized for quantitation of CYP1B1. *CYP1B1* and in parallel *CYP1A1* expression was determined in 30 specimen. CYP1B1 transcript levels ranged from 1.5 to 99. CYP1A1 levels had an even larger interindividual range. In most specimen *CYP1B1* expression was 2-6 fold that of *CYP1A1*. *CYP1B1* expression was significantly higher in the breast cancer group than in the healthy control group.

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Regine Goth-Goldstein 9/16/99

PI Signature Date

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Interindividual Differences in Metabolism of Carcinogens as a Risk Factor for Breast Cancer

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment from incomplete combustion of fossil fuels and other industrial sources. Coal tars and chimney soot, complex mixtures containing PAHs, were among the first substances ever to be associated with the development of tumors in exposed humans and animals. The major pathway by which ingested or inhaled PAHs are metabolized, is the stepwise oxidative activation by the cytochrome P450 isozymes, CYP1A1 and CYP1B1, followed by detoxification by phase II enzymes (1). The highly reactive intermediate formed by CYP1A1 or CYP1B1 can bind to DNA, the resulting DNA adduct can cause a mutation that if in a relevant gene could initiate cancer. Expression of both *CYP1A1* and *CYP1B1* is highly inducible by PAHs and other environmental toxins, such as dioxin (2).
Whereas CYP1A1 has been studied extensively for over 25 years, much less is known about CYP1B1, one of the newest members of the P450 family. There is considerable evidence that CYP1B1 could be a key enzyme in the activation of carcinogens in the breast and therefore play a role in the development of breast cancer. The *CYP1B1* gene is highly expressed in human breast tissue, but not in liver which has been considered the major site for metabolism of xenobiotic compounds (3). The CYP1B1 enzyme is involved in the activation of a number of lipophilic environmental carcinogens, including PAHs and aromatic amines, and in addition it hydroxylates 17 β-estradiol at the C-4 position to the potentially carcinogenic 4-hydroxy estradiol (4, 5). We are testing the hypothesis that individuals with higher levels of CYP1B1 are at a higher risk for breast cancer because they produce higher amounts of ultimate carcinogen. The expression level of *CYP1B1* is being determined in a collection of normal breast tissue samples from reduction mammoplasties and from mastectomy patients and *CYP1B1* expression is compared in specimen from cancer patients and healthy controls to establish if breast cancer patients have an increased level of the enzyme.

Body of Annual Report

Interindividual variation in carcinogen metabolism has been recognized as an important determinant of susceptibility to various cancers (6,7). The interindividual variability in the level of expression of phase I and phase II enzymes is due in part to genetic polymorphism. Several genetic polymorphisms have been described for CYP1A1 that can affect inducibility (8). Recently two genetic polymorphisms in CYP1B1 have been identified which seem to result in a considerable change of the enzyme activity (9, 10). Besides genetic background, various factors can modify expression of *CYP1A1* and *CYP1B1* in an individual, including hormonal levels, dietary and smoking habits, and exposure to other foreign compounds that act as inducers or repressors.
We are testing the hypothesis that the level of enzymes with the capacity to activate or detoxify environmental carcinogens in the breast represent a risk factor for breast cancer,

and specifically that individuals with higher levels of CYP1B1 are at a higher risk for breast cancer because they produce higher amounts of the ultimate carcinogen. Interindividual variations in the level of *CYP1B1* can be due to either polymorphism in the structural gene, polymorphism in a regulatory gene or finally due to environmental compounds that modify the expression of the enzyme by interacting with the Ah receptor. Since expression is the result of these various factors, studies on genetic polymorphism capture only a fraction of the enzyme variability in at risk individuals. We therefore decided to determine expression of *CYP1B1* in the breast to capture all possible modifying factors. A collection of histologically normal breast tissue specimens from mastectomy patients and from reduction mammoplasties is being analyzed. The goal for the initial 18 months of this project is to compare expression of *CYP1B1* in healthy individuals and breast cancer patients.

1. Method for measuring *CYP1B1* expression

The first task was to set up a quantitative RT-PCR assay for quantitation of CYP1B1 transcript by modifying an assay previously developed in this laboratory for quantitation of CYP1A1 relative to a constantly expressed gene (β-actin). We optimized the PCR reaction conditions to amplify a CYP1B1 fragment using previously published primers (3) and determined the range of linearity for the CYP1B1 amplification reaction. Quantitation by PCR is only accurate when the reaction remains in the exponential phase of product accumulation. Also, for relative comparison of different PCR targets, it is essential that the reaction efficiencies of the two targets is similar. We investigated the possibility of using a multiplex quantitative RT-PCR assay to quantify CYP1A1 and CYP1B1 transcripts simultaneously, but the optimal conditions for each target are so different that the reactions occurred with different and low efficiencies which make a simultaneous quantitation impossible.

We have succeeded in developing an assay that measures CYP1B1 expression in parallel to CYP1A1. The reactions occur in separate tubes which allows for optimal conditions and similar reaction efficiencies for each target. (CYP1A1 reaction uses 50 pmole of each primer and 6 mM $MgCl_2$ while the CYP1B1 reaction used 40 pmole of each primer and 2 mM $MgCl_2$. Each reaction takes place for 20 cycles using the same annealing temperature therefore, the reactions may take place in the thermal cycler at the same time.) To test the assay, we purified CYP1A1 and CYP1B1 PCR products, diluted them and mixed them together in known ratios before reamplification. Ratios of 100 to 1, 10 to 1, 5 to 1, 3 to 1, 1 to 1 (and the reverse) were made and then quantified by our assay. Relative signal intensities of CYP1A1 to CYP1B1 were as expected from the known mixtures. This test of the assay shows that there is no preferential amplification of one target over the other and that we can discriminate 3-fold differences in expression.

2. Expression of *CYP1B1* in breast tissue specimens

The second task, to determine the expression of the *CYP1B1* gene by this assay in a collection of normal breast tissue specimens from mastectomy patients and from reduction mammoplasties, has been initiated and the first 30 samples have been tested measuring in parallel *CYP1B1* and *CYP1A1* expression. *CYP1A1* expression had been measured previously in the same samples as part of another study. The repeat measurements gave very similar results (90 % of samples varied by less than a factor of

Table I: Expression of *CYP1B1* and *CYP1A1* in normal breast tissue of breast cancer patients and healthy controls

Tissue Source	CYP1A1/ Actin	CYP1B1/ Actin	CYP1B1/ CYP1A1
Reduction mammoplasties	5.2	3.9	0.8
	4.0	3.7	0.9
	3.8	9.9	2.6
	4.7	15.2	3.2
	5.4	21.9	4.0
	3.4	11.7	3.5
	0.2	8.9	42.0
	6.7	41.1	6.2
	0.18	7.1	40.6
	4.9	23.4	4.8
	31.4	7.1	0.2
	12.8	40.1	3.1
	1.2	12.8	10.5
	4.5	19.8	4.4
	9.4	26.4	2.8
	11.4	9.7	0.9
	6.4	13.1	2.0
	2.0	10.5	5.3
Mastectomies	13.3	58.2	4.4
	66.8	70.7	1.1
	25.0	62.9	2.5
	9.4	30.4	3.2
	5.1	98.6	19.3
	0.2	11.4	47.0
	4.1	27.9	6.8
	0.4	12.4	29.6
	16.2	63.6	3.9
	1.7	1.5	0.9
	2.2	11.5	5.1

2). This confirms that the method provides reliable results. In the specimen analyzed so far *CYP1B1* levels relative to actin ranged from 1.5 - 99 and *CYP1A1* levels ranged from 0.18 - 67. The results summarized in Table I show that CYP1B1 is expressed at higher level than CYP1A1 in most samples. For 17 of 29 samples the ratio of CYP1B1 to CYP1A1 is between 2 and 7, for 6 samples it is below 2, and for 6 samples it is between 10 and 50. These imbalances will be the subject of future studies. The findings indicate that CYP1B1 is the primary enzyme for PAH metabolism in the breast and might therefore have a role in PAH-carcinogenesis.

The most exciting conclusion from these results is that more specimen with high *CYP1B1* expression are among the breast cancer patients than among healthy controls. The values were analyzed by Student's t-test. Whereas the difference in CYP1A1 values between the study groups did not achieve statistical significance ($p = 0.1984$), the difference in CYP1B1 values between the study groups was statistically significant ($p = 0.0046$). The finding supports our hypothesis, that individuals with higher levels of CYP1B1 are at a higher risk for breast cancer. The study groups were small and we plan to expand the sample size of both groups in the coming year.

Key Research Accomplishments

1. A method has been developed to measure *CYP1B1* expression in parallel to *CYP1A1* expression.

2. This method was used to determine *CYP1B1* and*CYP1A1* expression in 30 normal breast tissue specimen. The results showed that in most specimen *CYP1B1* was expressed at considerably higher levels than *CYP1A1* , indicating that the CYP1B1 enzyme is primarily responsible for PAH activation in breast tissue.

3. *CYP1B1* expression is significantly higher in the breast cancer patients than in healthy individuals.

Reportable Outcomes

The findings will be presented at the California Breast Cancer Research Symposium. September 17 - 18, 1999 in Los Angeles as part of an invited talk on 'Metabolism of Environmental Chemicals as Breast Cancer Risk' where I will primarily describe an earlier competed study on CYP1A1 expression and genotype.

Conclusions

Because of the potential important role of CYP1B1 in the activation of environmental and endogenous compounds to carcinogenic intermediates, it was hypothesized that high CYP1B1 expression could represent a risk factor for breast cancer. This is the first study to measure expression of *CYP1B1* in a collection of normal breast tissue from mastectomy patients and from reduction mammoplasties, to estimate the interindividual variation of CYP1B1 levels and to compare expression in breast cancer patients and healthy individuals. The preliminary results show a large interindividual variation in *CYP1B1* expression and indicate that CYP1B1 is the predominant PAH-metabolizing enzyme in the breast. Finally, in the as yet small number of specimen analyzed, *CYP1B1* expression was higher in the breast cancer group compared to the control group and the difference was statistically significant.

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